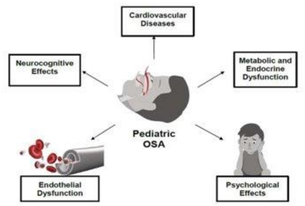
Pediatric Obstructive Sleep Apnea is Associated with Localized Loss of Immune Tolerance in the Tonsils

Thomas Hoag, Rajeev Aurora, PhD, Thomas Sanford, M.D.

SAINT LOUIS UNIVERSITY — EST. 1818

Introduction

- Obstruction of the pediatric airway is in part thought to be the result of tonsillar hypertrophy
- Although there is a strong link between tonsillar hypertrophy and pediatric OSA, the pathogenesis of tonsillar hypertrophy in children is poorly understood
- Tonsillar hypertrophy may be related to a process of dysregulated immune cell signaling and inflammation.

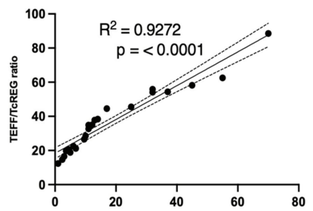


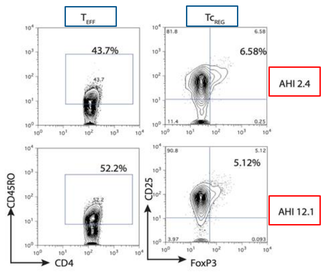
Hypothesis

Loss of immune tolerance in the pediatric tonsil leads to decreased downregulation and an increased proinflammatory state, ultimately causing tonsillar hypertrophy and worsened OSA.

Background- TEFF & TcREG

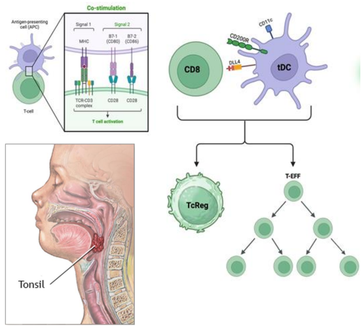
There are increased levels of TEFF cells (CD45+ CD3+ CD45RO+) and decreased levels of TcREG by flow cytometry with increasing AHI.





Mechanism of Interest

- Interaction between tDCs and CD8 T cells leading to induction of T-regulatory cells and T-effector cells.
- Key elements of investigation involved analysis of dendritic cell markers CD11C, as well as CD200 glycoprotein receptor and DLL4 notch ligand.



Methods

Analyzed three cohorts of patients for initial experiments
AHI and BMI were controlled in all patients

Cohort #	n	Indication
Cohort 1	16	OSA
Cohort 2	10	Recurrent Tonsillitis
Cohort 3	6	Non-OSA

Background – Flow cytometry: TEFF & TcREG cells in patients with low vs high AHI.

Figure 1 – Flow cytometry: Dendritic cells in patients with low vs high AHI.

Figure 2 – Co-culturing: tDC induction of TcREG: 4 days of co-culturing tDCs with naïve CD8 Tcells → Anti-CD3 antibody crosslinked with TCR → Induction of TcREG measured in patients with OSA vs no OSA.

Figure 3 – Single Cell RNA Sequencing comparing cell types in low vs high AHI.

Two additional patients were collected for staining
BMI was controlled in both patients

Patient #	AHI	BMI
Patient 1	3.9	17.3
Patient 2	39	16.5

T-cells – Immunohistochemistry Staining: Comparison of the % Area of CD3+ T-cells between low and high AHI. Comparison of the number of FoxP3+ cells/15x mag field.

T+DC – Immunohistochemistry Staining: Comparison of the % Area of overlap between CD3+ T-cells and CD11c+ dendritic cells between low and high AHI.

Dendritic Cells (DC) – Immunohistochemistry Staining: Comparison of the % Area of CD11c+ dendritic cells between low and high AHI.

Results

Immunohistochemical staining – TcREG &Teff Cells

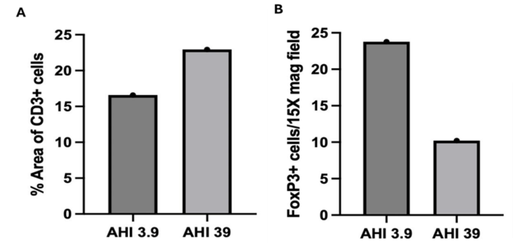
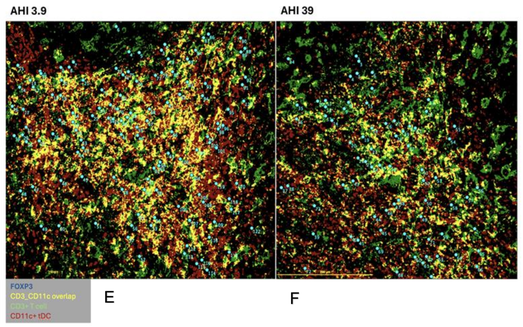
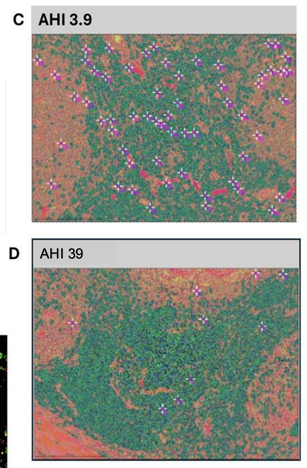


Figure A represents % area of CD3+ cells between low and high AHI patients. Figure B represents the number of FoxP3+ cells between low and high AHI patients. Images C and D are visual representations of CD3+ cells (green) with FoxP3 TcREGs overlayed (magenta dot with white crosshairs)





Figures E and F show colocalization at 15x mag between CD3+ T-cells and CD11c+ tDC (yellow) and the number of FoxP3+ TcREGs (Blue dots) with decreased overlap in higher AHI.

Immunohistochemical staining – T-cells and DC Overlap

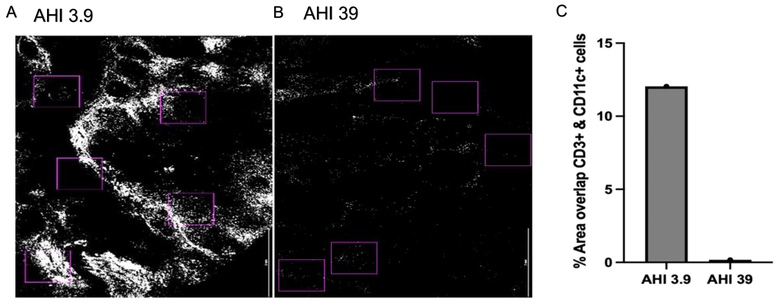


Image A shows colocalization between CD3+ T-cells and CD11c+ tDC for the entire tonsillar cross section. Figure B is a graphical representation of the % area overlap shown in Image B.

Immunohistochemical staining – Dendritic Cells

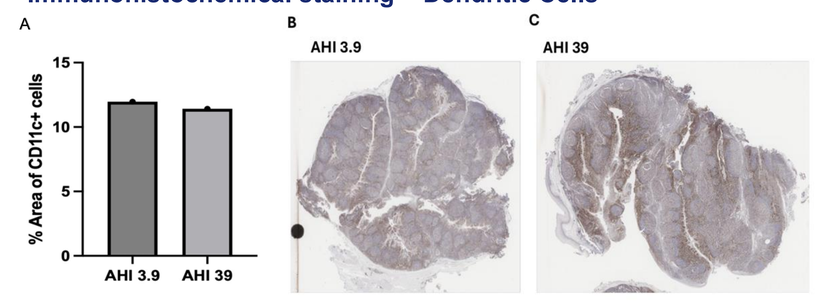
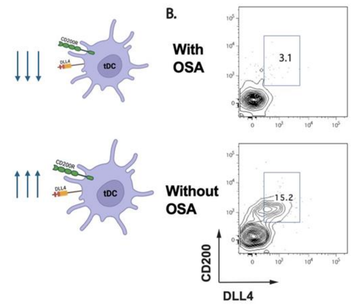


Figure A represents flow cytometry and shows no difference in CD11c+ tDCs between low and high AHI. Images B and C are gross images of each patients tonsils stained with CD11c. Importantly, CD11c does not differentiate between DLL4+ or DLL4- dendritic cells. Dendritic cells localize to **different locations in tonsils from patients with higher AHI**

Figure 1 - Flow Cytometry - Dendritic Cells

There were increased tDC (CD200+ and Notch ligand DLL4+) - APCs known to induce TcREG (FoxP3) and CD25 in CD8 T-cells in patients without OSA



Levels of CD200 DLL4 double-positive subset of DC (tDC) negatively correlated with AHI

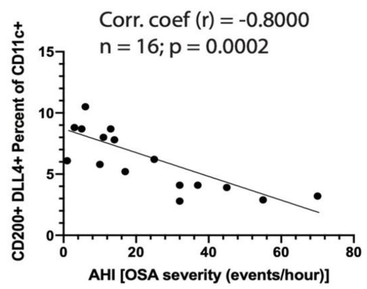
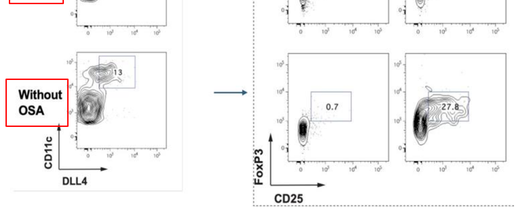
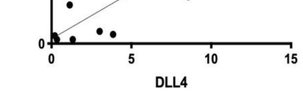


Figure 2 – tDCs induce TcREG

tDCs are more robust from patients with tonsillitis vs those with OSA.

These tDCs were more abundant after 4 days of co-culturing and ultimately led to higher numbers of TcREG induction.

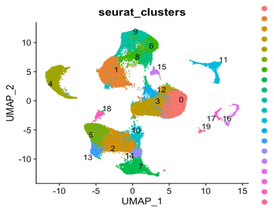




Relationship between levels of TcREG induction and DLL4+ DC expression

Figure 3 - scRNA-Seq

Single cell sequencing showed significant expansion of effector T-cells (cluster 2), germinal center B-cells (cluster 3), and smooth muscle cells (cluster 9). There were decreased but not statistically significant levels of FoxP3+ T-cells in patients with higher AHI.



Cluster#	Cell Type	AHI 5.5	AHI 31
0	Activated B-cells	15.9%	8.8%
1	T-cells CD45RO+ (memory T-cells)	7.4%	7.1%
2	T-cells CCR7 (effector T-cells)	5.7%	18.0%
3	Germinal ctr B-cells	3.8%	10.3%
4	B-cells	7.2%	9.5%
5	T-cells FoxP3+	5.7%	5.2%
6	Smooth muscle cells (SMC)	3.1%	8.7%
7	CD8 T-cells CD45RO+	8.3%	5.1%
8	Neutrophils	1.6%	8.5%
9	SMC - proliferating	2.5%	6.0%
10	B-cells - proliferating	6.5%	4.2%
11	Macrophages	1.5%	5.2%
12	NK cells	2.7%	1.9%
13	CD8 T-cells FoxP3+	2.7%	1.7%
14	CD8 T-cells	3.1%	0.4%
15	Plasmacytoid dendritic cells (pDC)	0.5%	3.8%
16-19	Fibroblasts	1.1%	1.8%

Conclusion

The results of flow cytometry and scRNA-seq indicate there is significant expansion of T-cells, B-cells, and smooth muscle cells along with a decrease in the production of FoxP3+ cells. This result is supported by our immunohistochemistry analysis which showed increase CD3+ T-cells and decreased FoxP3+ T cells in higher AHI patients. There was decreased overlap between CD11c+ DCs and T-EFF cells. Although we did not see a difference in the overall number of DCs by flow cytometry or IHC staining, there may be a difference in the spatial distribution of CD11c+ DCs that affects the interaction of DCs with T-cells.
Although we observe a loss of tDCs with increased AHI, We have not yet identified the mechanism promoting increased TEff cells. The Teff may be activated by an alternative APC pathway eg. B cells, leading to proinflammatory T-cell expansion. This represents an avenue of future study.